TITLE OF THE INVENTION
LIVE COCCIDIOSIS VACCINE

BACKGROUND OF THE INVENTION

It is known that infective stages of coccidial organisms, such as sporulated oocysts, can be administered to neonatal fowl to induce immunity against coccidiosis. However, administration of such "wild-type" organisms must be followed by the administration of anticoccidial agents to prevent the outbreak of coccidiosis by the infective organisms. In addition, the administration of such infective strains requires that the fowl, preferably chickens, be maintained on litter to provide for a constant level of reinfection to stimulate lasting immunity. This technique also suffers failure when too many or too few oocysts are administered. Being infective, too many oocysts can produce an actual outbreak of coccidiosis while too few oocysts result in inadequate levels of immunization leaving the chickens susceptible to natural outbreaks of coccidiosis.

One attempt at avoiding the problems associated with live vaccines is to administer attenuated precocious strains of Eimeria. These strains are less infective than the "wild-type" and tend to avoid problems associated with vaccines based on such strains. "Attenuated" strains are those that have been serially passaged through the host or through eggs, or a combination of host and egg passages, so that the resultant strain is less infective than the parent strain. "precocious" strains are those that have been developed to have short prepatent periods, which is the interval between the ingestion of an infective stage and its development within the host leading to the excretion of the next infective stage of the parasite. Attenuated strains are capable of conferring immunity and precocious strains are capable of developing into the next stage, however, the reduced level of infectivity of the attenuated strains, combined with the shortened development period of the parasite induces immunity without pathology and thus the host will not suffer ill effects from the vaccination.

However, such attenuated precocious strains, because they are less infective must be administered at higher levels, based on the number of oocysts administered, and it is recommended that vaccination with sporulated oocysts not take place earlier than three days after birth. See U.S. Patents 4,438,097 to Shirley and 5,055,292 to McDonald, et al.

SUMMARY OF THE INVENTION

The instant invention provides for the full immunization of fowl against coccidiosis by the oral administration of live sporulated oocysts of attenuated precocious strains of Eimeria at day one of age without the subsequent administration of anticoccidial agents. Thus, it is an object of this invention to describe the administration of such live, infective, but attenuated and precocious oocysts to newly hatched fowl. It is a further object to describe the effects such administration has upon the ability of such fowl to withstand a challenge of coccidiosis. A still further object is to describe formulations and methods to administer the oocysts to such fowl. Further objects will become apparent from a reading of the following description.

DESCRIPTION OF THE INVENTION

This invention is concerned with the administration of live, attenuated, precocious strains of coccidial organisms, in particular strains of Eimeria, to newly hatched fowl, in particular, chickens, but also turkey, ducks, quail and the like. The vaccine may be univalent, with a single species of Eimeria in the vaccine, but preferably a polyvalent vaccine is employed containing from two to all seven of the common species of Eimeria, viz E. necatrix, E. aservulina, E. brunetti, E. mitis, E. mivati, E. praecox and E. tenella. The particular content of the polyvalent vaccine, may be adjusted to contain different species depending on local conditions and the prevalence of particular species in certain areas. The most preferred vaccine contains all seven precocious species of Eimeria in the live attenuated form of sporulated oocysts.

The vaccine is administered to newly hatched chicks and preferably within one to two days of hatching. Following typical

husbandry techniques found in modern hatcheries, the vaccine is most preferably administered when the chicks are first processed in a process which administers other materials to the chick, such as other vaccines, for example Marek's disease, and perhaps vitamins and nutrients. During the process the chicks are also usually debeaked. Thus, the instant vaccine finds its most preferred industrial utility when administered along with other administered materials such that the chicks are only handled once. However, on farms with a smaller scale of operations, it will not be uncommon to administer the instant vaccine as a single administration without the concurrent administration of other materials and the simultaneous debeaking of the chicks.

The instant vaccine is generally administered as an aqueous suspension of the attenuated, precocious oocysts of Eimeria. To prevent the settling of the oocysts, the aqueous suspension can contain various suspending agents, thickeners and preservatives to assure an even distribution of the oocysts throughout the suspension.

The dosages of the attenuated precocious oocysts ranges from about 5 to about 1000 oocysts per bird for each of the Eimeria species included in the vaccine since one species will not necessarily confer immunity against a challenge from another species. A more preferred dosage will be from about 10 to 500 oocysts per species per bird, most preferably between 10 and 100 oocysts per species per bird. The precise number of each species present in the polyvalent vaccine will vary depending on the specific level of infectivity of each species and it will be normal to have a vaccine with different numbers of each species to reflect such different characteristics of each species. Generally, the vaccine is administered in a total volume of from about 0.1 to 1.0 ml, preferably about 0.25 to 0.50 ml.

The techniques for the preparation of the attenuated precocious strains of the different species of Eimeria are well known and utilize the procedure of serial passages of the coccidial strain through the host fowl or in embryonated eggs, or a combination of passages in fowl and eggs. Examples of such egg and fowl passages to

produce attenuated precocious strains is found in U.S. 4,438,097 to Shirley and U.S. 5,055,292 to McDonald, et al.

The vaccine discussed in McDonald, et al has been developed into a commercial product for chickens under the trademark of PARACOX. This is a combination of all seven principal species of Eimeria in an aqueous suspension. The product is labeled for administration to chickens from 5 to 9 days of age. Administration to chickens younger than 5 days is not recommended.

It will be appreciated by those skilled in the art that other strains of Eimeria exist which infect other animals than fowl, for example coccidiosis of cattle (E. zuernii, E. bovis) coccidiosis of swine (E. debliecki, E. neodebliecki, E. seabra and E. spinosa) as well as many other animals both avian and mammalian. Vaccines developed for such animals, based upon the use of attenuated precocious strains and infective stages of the parasite, can be used to impart long-term immunity to the parasite.

The following example is provided in order to more fully demonstrate the operation of the instant invention. It should not be construed as limitative of the invention.

EXAMPLE 1

A trial was conducted to examine the immunizing ability of different levels of precocious, attenuated oocysts (PARACOX, Pitman-Moore) given to one-day-old birds. Two thousand one hundred seventy six birds were assigned to 36 floor pens each containing 60 birds. In addition, 43 birds were maintained in batteries to be used as uninfected birds for challenge studies. The floor-pens were divided into four groups. Before placement in pens, birds of three groups were given oocysts by gavage equivalent to 0.2, 1 or 5 times the recommended number of oocysts in the labeling for PARACOX. For the next 27 days the birds were reared in their pens as usual with feed and water provided without restriction. The fourth group of birds was fed a ration containing 60 ppm of salinomycin to suppress coccidial

development; from Day 27 on these birds received unmedicated feed. Temperature and humidity were maintained at comfortable levels appropriate for the age of the chickens. When the birds were 27 days old, birds were taken from each pen, weighed and placed in battery cages. The birds remained on the same rations in cages as when on the floor. These birds were challenged with 10,000 sporulated oocysts of field strain of Eimeria tenella per bird after 24 hours in the cages. Feces were collected for Days 4 to 6 inclusive after challenge. The birds were weighed and necropsied at 8 days after challenge. The birds remaining in the floor-pens were weighed and challenged with 30,000 sporulated oocysts of Eimeria maxima per bird on Day 28. Samples of feces were collected from each pen at intervals to monitor oocyst output from the challenged birds. A sample of birds were taken at 7 days after challenge to determine lesion scores and all the birds were weighed.

The results of the study show that vaccinated birds were protected from the harmful effects of challenge (Tables 1 and 2) and that the birds showed significant weight gain and feed efficiency improvements over the unvaccinated birds. Table 3, 4 and 5 show the live weight of the birds, the average feed conversion per bird and the average feed efficiency per bird.

TABLE 1

Summary of weight gains for 8 days after challenge, lesion scores and oocyst output from birds challenged with Eimeria tenella in cages after vaccination at one day of age with a mixture of oocysts (PARACOX).

Variable	Control	Salino[1]	Oocysts, (Paracox dose)		
			0.2X	1X	5X
Weight gain, g	539	549	558	609	550
Lesion score	2.6	1.9	0.2	0.4	0.2
Oocyst, 10^6/bird	75	29	<.1	.1	<.1

[1]Salinomycin included in ration at 60 ppm.

TABLE 2

Summary of weight gains for 7 days after challenge, lesion scores and oocyst output of birds challenged with Eimeria maxima after vaccination at one day of age with a mixture of oocysts (PARACOX).

Variable	Control[1]	Oocysts, (Paracox dose)		
		0.2X	1X	5X
Weight gain, g	279	435	465	463
Lesion score	2.1	1.0	0.9	1.1
Oocyst, 10^6/bird	16288	2440	3087	3775

[1]Birds fed ration with 60 ppm salinomycin until Day 27 and unmedicated ration thereafter.

TABLE 3

Treatments were replicated eight times. Average Live Weight, Feed Conversion and, Feed Efficiency of birds vaccinated with 0.20X, 1X or 5X level of PARACOX administered once by gavage on Day 0 and compared with unvaccinated birds fed 60 ppm Salinomycin up to Day 27 and unmedicated feed thereafter.

Day 28

VARIABLE	PARACOX 0.2X	PARACOX 1X	PARACOX 5X	SALINOMYCIN 0-27 Days Unmedicated feed thereafter
Av. Live Weight (kg/bird)	1.085	1.085	1.080	1.077
Av. Feed Conversion g. of feed/ g. of weight gained	1.686	1.662	1.666	1.649
Av. of Feed Efficiency g. of weight gained /g. of feed	0.593	0.602	0.600	0.606

TABLE 4

Treatments were replicated eight times. Average Live Weight, Feed conversion and, Feed Efficiency of birds vaccinated with 0.20X, 1X or 5X level of PARACOX administered once by gavage on Day 0 and compared with unvaccinated birds fed 60 ppm Salinomycin up to Day 27 and unmedicated feed thereafter. Birds of all treatment groups were challenged on Day 28 with 30,000 sporulated oocysts of Eimeria maxima per bird via feed.

Day 35

VARIABLE	PARACOX 0.2X	PARACOX 1X	PARACOX 5X	SALINOMYCIN 0-27 Days Unmedicated feed thereafter
Av. Live Weight (kg/bird)	1.520	1.550	1.543	1.356
Av. Feed Conversion g. of feed/ g. of weight gained	1.808	1.769	1.773	1.865
Av. of Feed Efficiency g. of weight gained /g. of feed	0.553	0.565	0.564	0.536

TABLE 5

Treatments were replicated eight times. Average Live Weight, Feed Conversion and, Feed Efficiency of birds vaccinated with 0.20X, 1X or 5X level of PARACOX administered once by gavage on Day 0 and compared with unvaccinated birds fed 60 ppm Salinomycin up to Day 27 and unmedicated feed thereafter. Birds of all treatment groups were challenged on Day 28 with 30,000 sporulated oocysts of Eimeria maxiuma per bird via feed.

Day 42

VARIABLE	PARACOX 0.2X	PARACOX 1X	PARACOX 5X	SALINOMYCIN 0-27 Days Unmedicated feed thereafter
Av. Live Weight (kg/bird)	2.080	2.093	2.096	1.924
Av. Feed Conversion g. of feed/ g. of weight gained	1.891	1.865	1.865	1.920
Av. of Feed Efficiency g. of weight gained /g. of feed	0.529	0.536	0.539	0.521

WHAT IS CLAIMED IS:

1. A method for inducing immunity to coccidiosis in animals which comprises administering to such animal a vaccine composed of attenuated, precocious strains of sporulated oocysts of one or more species of Eimeria where the vaccine is administered orally upon birth or hatching of the fowl up to two days following the birth or hatching.

2. The method of Claim 1 where the vaccine is administered between birth or hatching of the animal and one day following the birth or hatching of the animal.

3. The method of Claim 1 where the animal being administered the vaccine is a fowl.

4. The method of Claim 2 where the fowl is chickens, turkeys, ducks or quail.

5. The method of Claim 3 where the fowl is chickens.

6. The method of Claim 1 where the vaccine is administered as an aqueous oral suspension of the sporulated oocysts.

7. The method of Claim 5 where the aqueous oral suspension also includes one or more suspending agents, thickeners or preservatives.

8. The method of Claim 1 where from 5 to 100 sporulated oocysts of each species of Eimeria are administered per animal.

9. The method of Claim 8 where from 10 to 500 sporulated oocysts of each species of Eimeria are adminstered per animal.

10. The method of Claim 9 where from 10 to 100 sporulated oocysts of each species of Eimeria are administered per animal.

11. The method of Claim 1 where the vaccine is composed of sporulated oocysts of one or more species of Eimeria selected from E. necatrix, E. acervulina, E. brunetti, E. maxima, E. mitis, E. praecox and E. tenella.

12. The method of Claim 11 where the vaccine is composed of sporulated oocysts of all of the species of Eimeria selected from E. necatrix, E. avervulina, E. brunetti, E. maxima, E. mitis, E. praecox and E. tenella.

INTERNATIONAL SEARCH REPORT

Int. ... application No.
PC.../US.../00075

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 39/00, 39/02; C12N 1/10, 1/36
US CL :424/88, 92; 435/245, 258
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/88, 92; 435/245, 258

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Abstract Avian Pathology, Volume 18, No. 2, issued 1989, Bedrnik et al, "Field Vaccination of Broilers Against Coccidiosis", Biodid znumber 88062625, pages 255-264, Abstract only.	1-12
X	Avian Pathology, Volume 15, issue 1986, Long et al, "Immunisation of Young Broiler Chickens with Low Level Infections of Eimeria tenella, E. acervulina or E. maxima", pages 271-278, see entire document.	1-12
X	US, A, 4,438, 097 (Shirley) 20 March 1984, Abstract, col. 4, lines 30-53; col. 5, lines 30-35.	1-12

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be part of particular relevance

"E" earlier document published on or after the international filing date

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"&" document member of the same patent family

Date of the actual completion of the international search
05 March 1994

Date of mailing of the international search report
MAR 28 1994

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/00075

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Second Asian/Pacific Poultry Health Conference, Proceedings 112, issued 23-25 September 1988, Shirley, "Control of Coccidiosis with Vaccines", pages 129-157, see pages 131-133, 135-136.	1-12